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The anionic amphiphile SDS is an antagonist for the human neutrophil formyl peptide receptor 1

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ABSTRACT

The anionic amphiphil sodium dodecyl sulfate (SDS) is commonly used to activate the superoxide-generating NADPH-oxidase complex in cell-free systems, but very little is known about the effects of SDS on intact cells. It was, however, recently shown that SDS causes a translocation and an activation of Rac (a small G-protein) in intact cells, but this signal is not in its own sufficient to activate the oxidase (Nigorikawa et al. (2004) [1]). We found that SDS acted as an antagonist for FPR1, one of the neutrophil members of the formyl peptide receptor family. Accordingly, SDS reduced superoxide anion production induced by the chemoattractant formylmethionyl-leucyl-phenylalanine (fMLF). The receptor specificity of SDS was fairly high, but the concentration range in which it worked was narrow. The length of the carbohydrate chain as well as the charge of the molecule was of importance for the antagonistic effects. Signaling through FPR2, a closely related receptor also expressed in neutrophils, was not inhibited by SDS. On the contrary, the response induced by the FPR2-specific agonist WKYMVM was primed by SDS. The precise mechanism behind the primed state is not known, but might be related to the effects earlier described for SDS on the small G-protein Rac, that is of importance for a proper transduction of the down-stream signals from the occupied receptor.

1. Introduction

Professional phagocytes of our innate immune system [2] produce oxygen radicals, which are essential for the defense against invading microbes [3,4]. The molecular basis for activation of the superoxide-generating NADPH-oxidase in response to for example chemoattractants is the binding of ligands to specific cell surface receptors [5]. The occupied receptors induce a variety of intracellular signals directly or indirectly responsible for activation of the oxidase, and the second or third messengers generated translocate the soluble (non-membrane bound) oxidase components $p47^{phox}$, $p67^{phox}$, $p40^{phox}$, and a small G-protein (Rac) from the cytosol to a b cytochrome-containing membrane [6–8]. The assembled oxidase then starts to ferry electrons from NADPH to molecular oxygen to generate superoxide anions. A great deal of our knowledge of the structural and functional properties of the individual oxidase components as well as of the assembly of the system comes from experiments performed in cell-free oxidase systems [9,10]. In such systems, arachidonic acid or anionic amphiphils such as sodium dodecyl sulfate (SDS) are routinely used to facilitate the formation of an active superoxide-generating complex [11,12], and the mechanisms suggested, include a direct conformational change of $p47^{phox}$ and possibly a phosphorylation of the same component. The effects of arachidonic acid on the NADPH-oxidase have been extensively studied also in intact neutrophils, and shown to be one of the most potent inducers of superoxide anions [13,14]. Even though SDS is a commonly used trigger of the NADPH-oxidase system in cell-free systems, few studies have addressed the impact of the amphiphile on the NADPH-oxidase in intact cells. It was, however, recently shown that the amphiphile alone causes a translocation of a small G-protein (Rac) from the cytosol to the plasma membrane as well as a conversion of this protein to its GTP-bound state [1]. The effects on Rac are obtained not only in the cell-free oxidase system but also in intact cells. In cells, this signal is not on its own sufficient to activate the oxidase [1], suggesting fundamental differences between arachidonic acid and SDS as triggers of NADPH-oxidase activity.

Ligand-binding to either one of the two neutrophil members of the formyl peptide receptor family (FPR1 and FPR2), results in activation of the NADPH-oxidase and cellular release of superoxide. The two receptors share a high degree of amino acid identity, but still, they are triggered by different agonists and different second messengers are generated by the activated receptors [15–17]. Whereas FPR1 is a high affinity pattern recognition receptor with ability to track bacteria releasing formylated peptides [18,19], FPR2 has a low affinity for formylated peptides. Instead a number of non-formylated peptides are recognized by and can activate the

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receptor [5,20,21]. Furthermore, the signaling routes used by the receptors differ as illustrated by the fact that a cell-permeable peptide with a sequence corresponding to the PIP_2-binding region of gelsolin [22], selectively blocks FPR2-mediated superoxide production and granule secretion [17]. Taken together, these data clearly demonstrate that there are fundamental differences in ligand-binding properties as well as in intracellular signaling between these two closely related members of the formyl peptide receptor family (for a review see [23,24]).

The aim of this study was to determine the indirect effects of SDS-induced Rac translocation and conversion to the GTP-bound state [1], on fMLF (a specific agonist for FPR1) and WKYMVM (a specific FPR2 agonist) triggered neutrophil NADPH-oxidase activity. We found that SDS inhibited the oxidase activity induced by fMLF but instead primed the WKYMVM triggered response. The SDS mediated inhibition of the fMLF response was due to an antagonistic effect of the detergent. The priming effect is suggested to be due to interference with the signaling part of FPR2.

2. Material and methods

2.1. Isolation of human neutrophils

Neutrophil granulocytes were isolated from buffy coats obtained from apparently healthy adults. After dextran sedimentation at $1 \times g$, hypotonic lysis of the remaining erythrocytes, and centrifugation in a Ficoll-Paque gradient, the neutrophils were washed twice and resuspended (1×10^7/ml) in Krebs-Ringer phosphate buffer containing glucose (10 mM), Ca^{2+} (1 mM), and Mg^{2+} (1.5 mM) (KRG; pH 7.3). The cells were kept on melting ice and used within 120 min of preparation.

2.2. Neutrophil NADPH-oxidase activity

Neutrophil superoxide anion production was determined using an isoluminol-enhanced chemiluminescence (CL) system [25,26]. The CL activity was measured in a 6-channel Biolumat LB 9505 (Berthold Co, Wildbad, Germany) using disposable 4-ml polypropylene tubes with a 1-ml reaction mixture. Tubes containing isoluminol (2×10^{-5} M), horseradish peroxidase (HRP, 2 U), and neutrophils (10^6/ml) were allowed to equilibrate for 10 min at 37 °C, after which 0.1 ml of stimulus was added and the light emission was recorded continuously (details about the CL technique are given in [26]).

2.3. Determinations of receptor exposure by FACS analysis

To determine the effect of SDS on ligand-binding to FPR1, a FITC-conjugated formylated hexa peptide (FITC-fNLPNTL; 10^{-9} M final concentration) and a Cy5-conjugated hexapeptide (Cy5-WKYMVM; 10^{-9} M final concentration) were added to neutrophils on ice. The peptides were added to neutrophils in the absence or presence of SDS or non-labeled fMLF/WKYMVM (10^{-7} M). The cells were then incubated at 4 °C for 30 min, and no washing was performed after labeling. The amount of specifically bound probes was determined as the mean fluorescence intensity (MFI) using an Accuri C6 flow cytometer equipped with two laser lines (488 and 640 nm; Accuri Cytometers Ltd. Camps, UK).

2.4. Calculation of contact angles

The effect of SDS on the surface tension was determined by contact angle measurements as described earlier [27]. In short, 10 μl drops of KRG containing different concentrations of SDS were placed on an ordinary polystyrene Petridish and the contact angles at the liquid/solid/air meeting point were calculated from the diameter of the drops.

2.5. Chemicals

Isoluminol and fMLF, phorbol myristate acetate (PMA), sodium dodecyl sulfate (SDS) as well as the other detergents used were obtained from Sigma (St. Louis, MO). IL-8 was from R&D systems (Minneapolis, MN) and platelet activating factor (PAF) was from Avanti Polar Lipids (Alabaster, AL). The hexapeptides Trp-Tyr-Met-Val-L/DMet-NH2 (WKYMVM/m) were synthesized and purified by HPLC by Alta Bioscience (University of Birmingham, Birmingham, United Kingdom). The annexin I-derived peptide Gln^9-Lys^{25} [28,29] and the formylMet-Ile-Phe-Leu (fMIFL) peptide were synthesized and purified by HPLC by Ross-Petersen ApS (Holte, Denmark). The FITC-labeled formyl-Nle-Leu-Phe-Nle-Tyr-Lys (FITC-fNLFNTL) was from Molecular Probes and the Cy5-labeled Trp-Tyr-Met-Val-Met-NH2 (Cy5-WKYMVM) peptide was from Phoenix Pharmaceuticals (Burlingame, CA, USA). Catalase and horseradish peroxidase (HRP) were purchased from Boehringer-Mannheim (Mannheim, Germany). Dextran and Ficoll-Paque were from Pharmacia (Uppsala, Sweden). The peptide QRLFQVKGRR (gelsolin residues 160–169) coupled to rhodamine (PBP10) was a generous gift from Paul Janmay (Philadelphia, PA, USA).

3. Results

3.1. Neutrophil NADPH-oxidase activity induced by fMLF and WKYMVM and the effect of SDS

The two chemoattractant peptides fMLF and WKYMVM both induced a robust neutrophil oxidative burst with very similar time courses (Fig. 1). A very short lag phase is followed by a rapid increase of superoxide release and a peak of activity is reached after around 1 min. Not only the time course is very similar for the responses induced by the two peptides, but they also trigger a response that is of the same magnitude. The EC_{50} values differed, however, being 2×10^{-8} M and 4×10^{-8} M for the FPR1 agonist fMLF and the FPR2 agonist WKYMVM, respectively.

We have earlier shown that the magnitude of the fMLF and WKYMVM responses (despite the fact that well established and standardized isolation and storage protocol are used), displays a variation by a factor 50 when cells from different blood donors are used, but the ratio between the two responses with neutrophils from one individual is always close to 1 [30]. When SDS was added to the system in a certain concentration interval, the ratio between the response induced by fMLF and WKYMVM was reduced and the ratio varied from around 0.05 to 0.3. These results suggest selectivity in the sensitivity to the detergent. In quantitative terms the fMLF-induced neutrophil ROS production was reduced concentration dependently and at higher concentrations of SDS, also the WKYMVM response was reduced (Fig. 2). The shift in the fMLF/WKYMVM response ratio was not due only to inhibitory effects on the fMLF-induced response, but also to the fact that the WKYMVM-induced neutrophil production of superoxide was increased by SDS (Fig. 2). The precise concentration of SDS needed for the dual effect differed from day to day, but the concentration that reduced the fMLF response by 80% always primed the WKYMVM response. The IC_{80} of SDS (the concentration needed to obtain an 80% inhibition of the fMLF-induced response; hereafter denoted as SDS-IC_{80}^{fMLF}) was determined each experimental day and was generally around 60 μM (61 ± 32 μM; mean ± SD, $n = 18$). As determined by the change in contact angle in that concentration range of SDS, this was below the critical micelle concentration (CMC) of the detergent. At concentrations above the CMC the contact angle at the air/liquid/solid meeting point is constant [31]. Under our experimental conditions, the contact angle gradually decreased with increasing concentrations of SDS, being >85° at concentrations below 20 μM, 65° at 100 μM and 56° at 1 mM.

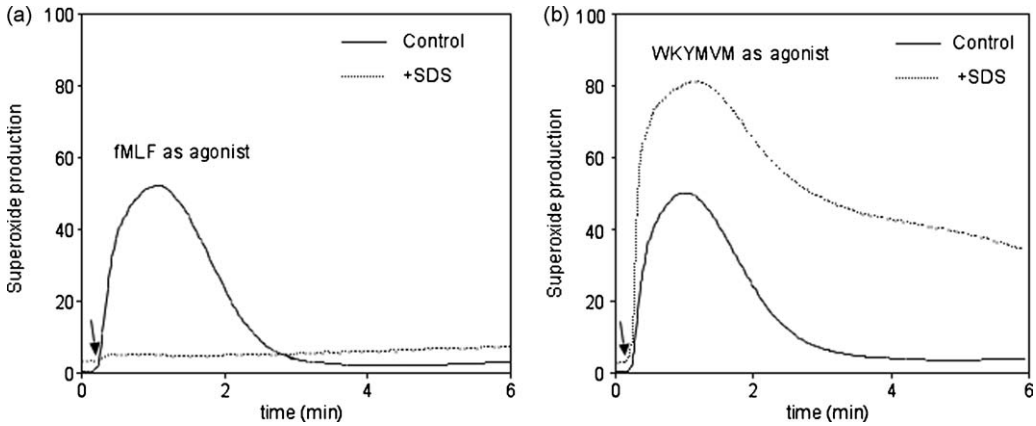

Fig. 1. Activation of the neutrophil NADPH-oxidase by FPR1 and FPR2-specific agonists and effects of SDS. Neutrophils were pre-incubated at 37 °C for 5 min without (solid lines) or with SDS (broken lines; 60 μM) and then challenged with fMLF (10^{-7} M final concentration; (a)), or WKYMVM (10^{-7} M final concentration; (b)) and the extracellular release of superoxide anion was monitored as light emitted in the isoluminol-amplified chemiluminescence system. The time point for addition of the agonist is indicated by an arrow and the amount of superoxide is expressed in arbitrary units. Abscissa; time of study (min); ordinate; superoxide production given as light emission and expressed in arbitrary units (cpm $\times$ 10^{-6}). One representative experiment out of more that 20 is shown.

The increase of the WKYMVM-induced response at the SDS-IC_{80}^{fMLF}, was more than 50% when determined as the differences between the peak values of the responses. The IC_{80} concentration of SDS for the fMLF-induced response was used also together with phorbol myristate acetate (PMA; a stimulus that bypasses the receptors and activate PKC directly), and we found that the PMA response in the presence of SDS was slightly increased compared to the control (ratio ± SDS = 1.2 ± 0.1; mean ± SD, n = 3).

3.2. Ligand and receptor specificity/selectivity for the SDS effect

The ligand dependency was determined using other agonists for FPR1 and FPR2. We have earlier shown that an annexin I-derived peptide triggers neutrophils through an interaction with FPR1 [28].

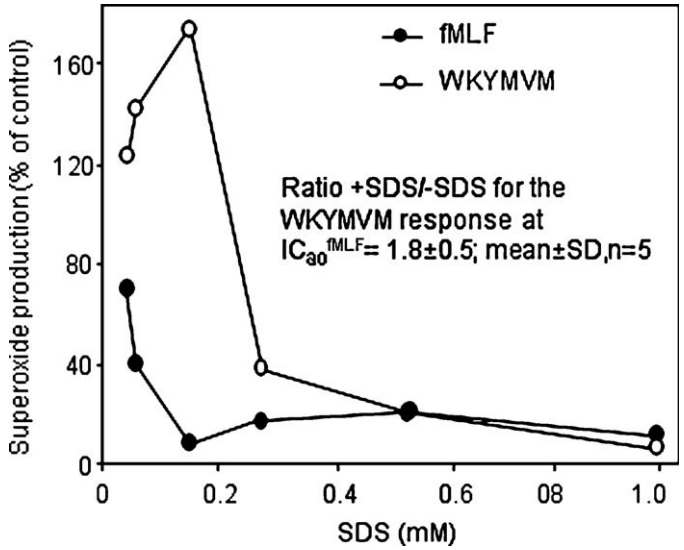

Fig. 2. SDS either inhibits or primes the neutrophil NADPH-oxidase activity when triggered by FPR1 and FPR2-specific agonists, respectively. Neutrophils were pre-incubated at 37 °C for 5 min with or without SDS (different concentrations) and then challenged with fMLF (10^{-7} M final concentration), or WKYMVM (10^{-7} M final concentration). The peak values of superoxide release in the presence of SDS was determined and compared to that induced without any detergent. Abscissa; concentration of SDS; ordinate; superoxide production given as percent of that obtained in the absence of detergent. One representative experiment is shown, and to show the reproducibility in the experimental set-up, the fold increase (ratio between peak value +SDS/−SDS) in the WKYMVM response is given.

When neutrophils were pre-treated with SDS the NADPH-oxidase response induced by the annexin I peptide was inhibited to the same level as the fMLF induced response, suggesting a selectivity for this receptor (data not shown).

A formylated tetrapeptide derived from *Staphylococcus aureus*, fMIFL (N-formyl-Met-Ile-Phe-Leu), earlier described to be a potent agonist for mouse neutrophils [32] was found to be a selective FPR1 agonist in human cells (data not shown). The SDS-IC_{80}^{fMLF} failed to affect the response induced by an equimolar concentration (10^{-7} M) of fMIFL (data not shown). It should be noticed, however, that the fMIFL peptide is much more potent that fMLF in activating the oxidase, having an EC_{50} value of 2×10^{-10} M compared to 2×10^{-8} M for fMLF, and the inhibitory effect of SDS was obvious also with fMILF when the concentration of the peptide used to trigger the cells was reduced (Fig. 3).

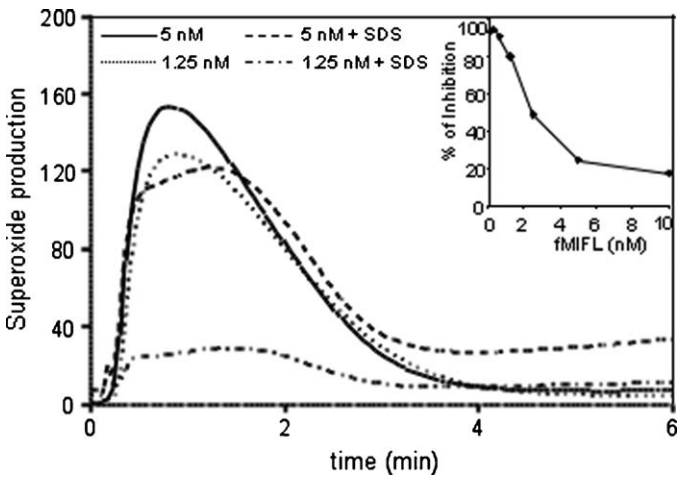

Fig. 3. Activation of the neutrophil NADPH-oxidase by the FPR1-specific agonist fMIFL and effects of SDS. Neutrophils were pre-incubated at 37 °C for 5 min with (broken lines) or without (solid lines) SDS. The concentration chosen inhibited the fMLF-induced activity by ≈80%, SDS-IC_{80}^{fMLF}. Neutrophils were challenged with the FPR1-specific agonist fMIFL at different concentrations. The extracellular release of superoxide anion was monitored as light emitted in the isoluminol-amplified chemiluminescence system. The amount of superoxide is expressed in arbitrary units. Abscissa; time of study (min); ordinate; superoxide production given as light emission and expressed in arbitrary units (cpm $\times$ 10^{-6}). Inset: abscissa; concentration of fMIFL; ordinate; superoxide production given as percent of that obtained with the same concentration of agonist but without any SDS. One representative experiment out of three is shown.

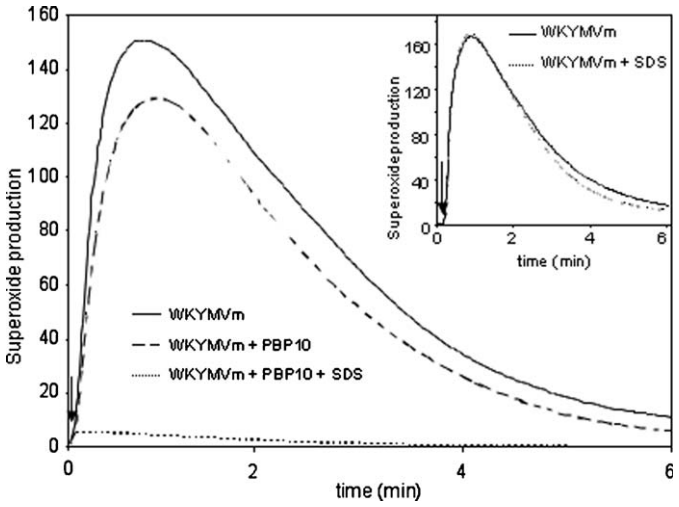

Fig. 4. Activation of the neutrophil NADPH-oxidase by an agonist with dual receptor specificities, WKYMVm, and effects of SDS. Neutrophils were pre-incubated at 37 °C for 5 min with or without SDS and in combination with the FPR2-specific inhibitor PBP10 (1 μM final concentration). The concentration of SDS chosen inhibited the fMLF-induced activity by ≈80%. Neutrophils ± SDS were challenged with the agonist WKYMVm and the extracellular release of superoxide anion monitored was unaffected by the detergent (inset). The sensitivity to SDS was changed when signaling through FPR2 was blocked by PBP10. The time point for addition of the agonist is indicated by an arrow and the amount of superoxide was determined as light emitted in the isoluminol-amplified chemiluminescence system. Abscissa; time of study (min); ordinate; superoxide production given as light emission and expressed in arbitrary units (cpm × 10^{-6}). One representative experiment out of three is shown.

The peptide WKYMVm in which the L-methionine in WKYMVM has been replaced with the D-isomer of the same amino acid, can bind and activate both FPR1 and FPR2 [15], but FPR2 is the preferred receptor. According to its receptor preference (FPR2) the response induced by WKYMVm was not affected (or in some experiments slightly primed) by SDS (Fig. 4 inset). The neutrophil response to WKYMVm is somewhat reduced in the presence of the FPR2-specific inhibitor PBP10 [17] but at the same time there is a receptor switch so that FPR1 becomes the preferred receptor when FPR2 signaling is blocked [33]. Accordingly, in the presence of PBP10 the WKYMVm-induced response was converted from being SDS insensitive to be fully inhibited in the presence of the detergent (Fig. 4).

Table 1
Summary of the effects mediated by SDS on neutrophil NADPH-oxidase activity when stimulated with different receptor specific GPCR agonists.

Agonist	Receptor	SDS effect
fMLF	FPR1	Inhibition
fMIFL (high con.)	FPR1	Not affected
fMIFL (low con.)	FPR1	Inhibition
WKYMVM	FPR2	Priming
WKYMVm	FPR2	Not affected
WKYMVm + PBP10	FPR1	Inhibition
IL-8	CXCR1/2	Not affected
Annexin I	FPR1	Inhibition
PAF	PAFR	Priming

Neutrophils express several other GPCRs (G-protein coupled receptors) including CXCR1 (receptor for the chemokine IL8) and PAFR (receptor for platelet activating factor). In order to determine the receptor selectivity of SDS, we investigated the effect of the detergent when other receptor ligands were used to trigger the neutrophil NADPH-oxidase. At SDS-IC_{80}^{fMLF} we found that the response induced by IL8, was not substantially affected by SDS. The response induced by PAF was, however, primed even to a higher degree than that induced by WKYMVM (Table 1).

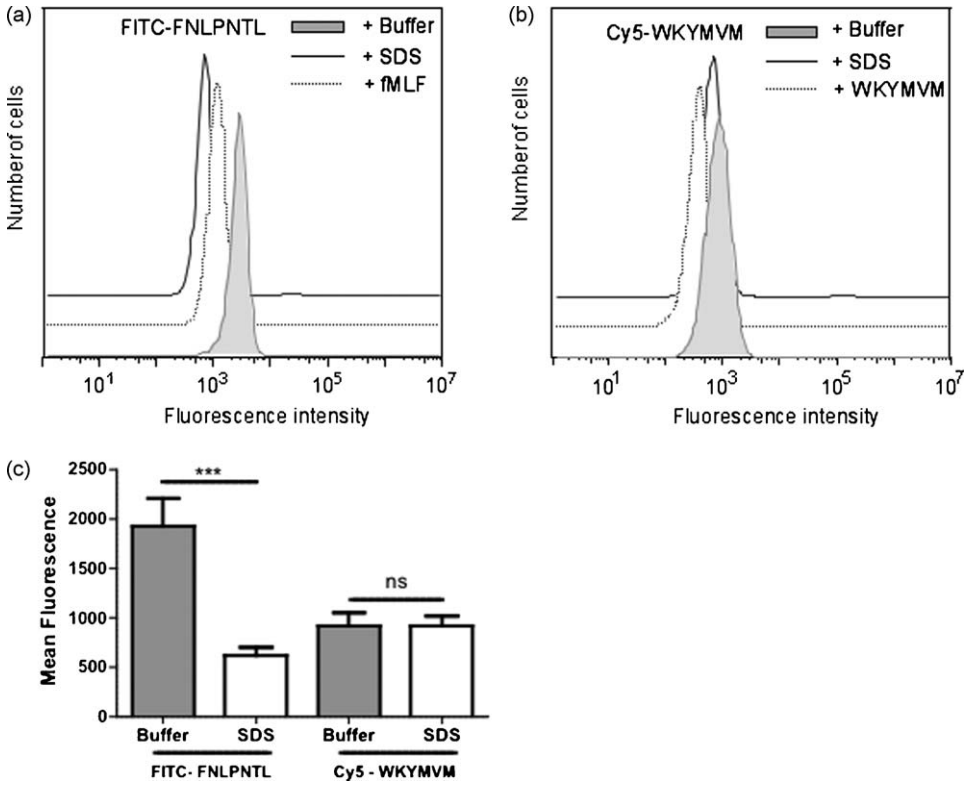

Fig. 5. Binding of the FPR1 agonist FITC-fNLFNTL to its cell surface receptor is reduced by SDS. Binding of an FITC-conjugated formylpeptide to neutrophils determined by flow cytometry. An excess of non-labeled fMLF reduced the binding of FITC-fNLFNTL to neutrophils (upper part; a); also SDS inhibited the binding of the formylated peptide (a and c; $p < 0.001$, ANOVA with Bonferroni's post-test). There was no significant effect of SDS on the binding of the Cy5-conjugated WKYMVM-peptide that binds to FPR2 (b and c; $p > 0.05$). The figure shows curves from a representative experiment (a and b) and MFI (mean fluorescence intensity)-values (±SD; $n = 3$; c).

3.3. SDS inhibits binding of a formylated peptide

Neutrophils were incubated for 5 min at 4 °C in the presence or absence of SDS-IC_{80}^{fMLF}. To determine the effect of SDS on peptide binding, a FITC-conjugated formylated peptide (FITC-fNLPNTL; 10^{-9} M final concentration) or a Cy5-labeled WKYMVM-peptide (10^{-9} M final concentration) was added and the amount of bound probe (mean fluorescence intensity; MFI) was determined by flow cytometry. The binding was specific, illustrated by the fact that an excess of non-labeled fMLF reduced the binding of FITC-fNLPNTL (Fig. 5a) whereas WKYMVM reduced binding of Cy5-WKYMVM (Fig. 5b). The presence of SDS reduced the neutrophil binding of FITC-fNLPNTL but not of Cy5-WKYMVM (Fig. 5).

3.4. The length and the charge matter

SDS (sodium lauryl sulfate or sodium dodecyl sulfate) is an anionic surfactant that has a tail of 12 carbon atoms attached to a sulfate group. The amphiphilic properties are retained also in molecules with a shorter or longer tail of carbon atoms. We determined the effects of molecules with a tail that was varied in length from 8 to 14 carbon atoms, and we found that at the SDS-IC_{80}^{fMLF} (with a tail of 12 carbon atoms) the degree of inhibition with the same molar concentration of the compound containing 8 or 10 carbon atoms was lower and somewhat higher with that containing 14 carbon atoms (Fig. 6a). These data suggest that the inhibitory effect increases as a function of the length of the tail of carbon atoms.

In order to determine the role of the charge we used a non-ionic detergent, octyl glucoside. No selective inhibition was obtained when SDS was replaced with octyl glucoside (Fig. 6b), suggesting that not only the length of the carbon chain but also the presence of a charged polar head is of importance.

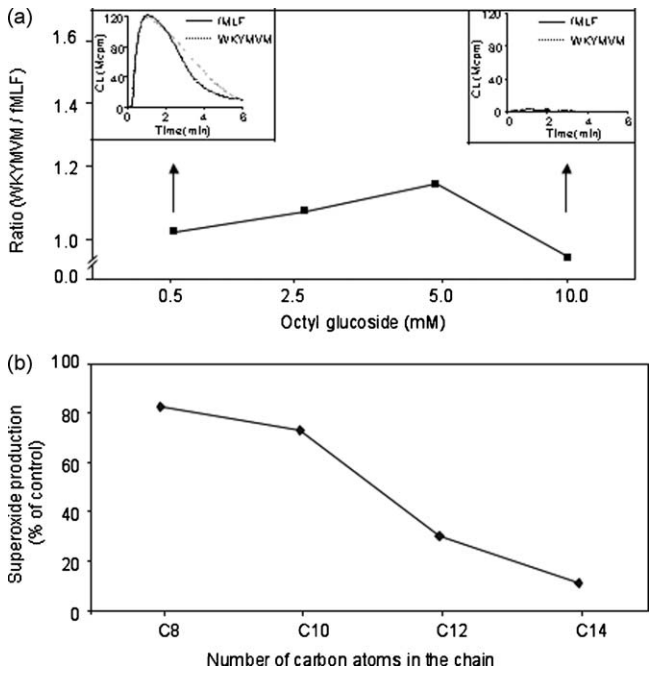

Fig. 6. Inhibitory effects of a non-charged detergent and of charged detergents differing in the length of the tail of carbon atoms. (a) Neutrophils were pre-incubated at 37 °C for 5 min with or without octyl glucoside (OG; different concentrations) and then challenged with fMLF (10^{-7} M final concentration), or WKYMVM (10^{-7} M final concentration). The peak values of superoxide release in the presence of OG was determined and compared to that induced without any detergent. Abscissa; concentration of OG; ordinate; ratio between the fMLF and WKYMVM-induced responses. One representative experiment out of three is shown and the magnitude and kinetics of the induced responses for two different OG concentrations (0.5 and 10 mM) are shown in the insets. (b) Neutrophils were pre-incubated at 37 °C with or without SDS-$IC80^{fMLF}$ or equimolar concentrations of "SDS variants" with shorter or longer carbon tails. After 5 min neutrophils were challenged with fMLF (10^{-7} M final concentration). The peak values of superoxide release in the presence of detergents were determined and compared to that induced without any detergent. Abscissa; number of carbon atoms in the chain; ordinate; superoxide production given as percent of that obtained in the absence of detergent. One representative experiment out of three is shown.

4. Discussion

In this study we found that SDS antagonizes FPR1 signaling. The effect of SDS was dependent on the length of the carbon chain (the longer the better) as well as of the charge, and the molecule blocked activation induced by all FPR1 agonists investigated. Due to the crucial roles of the FPR family receptors in inflammatory reactions as well as in regulation of pain [34,35], large efforts have been put into the search for receptor antagonists for the members of this receptor family. To date, only a couple of FPR2 antagonists have been identified [36], and a limited number of FPR1-specific antagonists have been described [37]. It is notable that many of these antagonists have no obvious structural similarities with known FPR1 or FPR2 agonists (for a review see [23]), an exception being the most commonly used ones. These antagonists have been developed by replacing the formyl group in bacterial derived peptides with a t-butyloxylcarbonyl (Boc) group. It is easy to imagine how this type of small change in one of the important binding domains of an agonist may transfer the molecule to an antagonist that competes in binding with the original molecule, but also cyclosporine H (CsH) has been shown to be a highly selective FPR1 antagonist [38]. There is no amino acid sequence similarity between the cyclic undecapeptide CsH and the antagonistic Boc peptides. This does not exclude the possibility that there might be similarities in the tertiary structure of the antagonists, but it might also be that they share important physico-chemical properties. The potent FPR1 antagonist Boc-PLPLP as well as CsH are very hydrophobic [38]. This is a property shared also by the amphipathic molecule SDS, but it is obviously not the only important property, as the hydrophobic but non-ionic detergent octylglycoside lacks the antagonistic properties of SDS.

The two formyl peptide receptors, FPR1 and FPR2, share a large sequence similarity, induce almost indistinguishable cellular responses, and there are agonists that can bind to both receptors. We have earlier shown that the hexapeptide WKYMVM selectively activates neutrophils via FPR2 [15]. This peptide therefore emerges as a very useful agonist to study this receptor, without interference emanating from the activation of FPR1. An exchange of the carboxyterminal L-methionine in WKYMVM for the D-isomer generates a peptide that increases its binding to FPR2 but at the same time the D-methionine containing peptide is an agonist for FPR1. This receptor is, however, not used unless signaling through FPR2 is blocked [33]. We used an earlier described FPR2-specific inhibitor (the gelsolin derived peptide PBP10) to determine the receptor preference for SDS. We show that the WKYMVm-induced neutrophil response was inhibited by SDS only when the FPR2 signaling pathway was blocked, suggesting a specificity of SDS for FPR1 and that activation by WKYMVm involves different parts (binding sites) on FPR1 and FPR2, one being SDS sensitive and the other insensitive. The same type of inhibition profile is obtained when the FPR1-selective antagonist CsH is used to inhibit the neutrophil response with WKYMVm as the agonist [33]; that is, CsH inhibits the response only when signaling through FPR2 is blocked.

Despite the large similarities between FRP1 and FPR2, the effect of SDS on the response triggered through the two receptors was totally different. The neutrophil response induced by the FPR2-specific agonist WKYMVM was primed, and this was true also for

the response induced by PAF. The molecular mechanisms behind priming of the NADPH-oxidase activity have been extensively studied and discussed [39–41]. The suggested mechanisms include alterations of intracellular signaling pathways (increased protein phosphorylation, phospholipase activity, intracellular Ca^{2+} changes and cross talk between Ca^{2+} increase and tyrosine phosphorylation), altered assembly of the NADPH-oxidase, proteolytic processing of cell surface proteins, and mobilization of new receptors to the cell surface on the primed cells. We have focused our efforts on the antagonistic effects of SDS, but it is possible that translocation of the small G-protein Rac from the cytosol to the plasma membrane that has been shown to be induced by SDS in intact cells, is a part of the priming process. The conversion of Rac to its GTP-bound state [1], might also be part of the priming process, but the precise mechanism remains to be determined. It should also be noticed that SDS is a highly effective surfactant, found in many household products such as toothpastes, shampoos, shaving foams, some dissolvable aspirins, enemas, and fiber therapy caplets. The presence of SDS is also linked to one of the most common oral conditions, a type of oral ulcer known as a canker sore (or aphtous stomatitis/Sutton's disease), which presents as a painful open sore inside the mouth or upper throat (including the uvula) [42,43]. A switch from an SDS-containing toothpaste to one that is free of this compound, reduces the amount, size and recurrence of aphthous ulcers. The same positive effects on ulcer development are obtained if SDS is replaced with another detergent [42,43], suggesting that it is not the surfactant activities but rather more specific/selective effect of SDS (such as those described here) that are of importance for the development of the aphthous ulcers. Taken together, we found SDS to be a selective FPR1 antagonist, and together with other selective/specific antagonists it may be used as a tool for further characterization of the FPR family in host defense and inflammation as well as to improve the possibilities to develop a new class of drugs that regulate the inflammatory process.

Acknowledgements

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